

## EXTERNAL SCIENTIFIC REPORT

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# Extensive literature search on *N*-nitroso compounds in food

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### Abstract

An Extensive Literature Search (ELS) by using PubMed, Web of Science and SciFinder for relevant studies on *N*-nitroso compounds (NOCs) analysed in food was performed. Search queries allowed to obtain a total number of 18592 non-redundant references. The relevance analysis of these references was performed by screening of title and abstract related to the chemical identity and characterisation, sources and occurrence in food, toxicokinetics and toxicity of NOCs applying eligibility criteria. Relevant references accounted for: i) Area 1 (Data on chemical identification and characterisation of NOCs found in food products) 176 documents; ii) Area 2 (Data on sources and occurrence in food, including human milk) 234 documents; Area 3 (Data on toxicokinetics, i.e. absorption, distribution, metabolism, excretion, in experimental animals and humans and from *in vitro* studies) 148 documents; Area 4 (Data on toxicity in experimental animals) 143 documents; Area 5 (Data on *in silico*, *in vitro* and *in vivo* genotoxicity and mode of action) 726 documents; Area 6 (Data on observations in humans including epidemiological studies, case reports, biomarkers of exposure) 333 documents. A total number of 1494 non-redundant relevant documents was found.

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**Key words:** Extensive Literature Search, *N*-nitrosamines, *N*-nitroso compounds, food

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## Summary

The overall purpose of this project was the identification and selection of relevant literature to gather information on chemical identification and characterisation, sources and occurrence in food as well as data on toxicokinetics and toxicity of *N*-nitroso compounds (NOCs) in order to support the preparatory work for the human health risk assessment of these substances. The project was developed in order to pursue three major objectives explicated below.

### *Objective 1*

A protocol for a tailored search strategy to retrieve studies including reviews and grey literature pertinent to the risk assessment and characterisation of NOCs analysed in food products was developed. The selection of keywords and the query syntax were customized for PubMed, Web of Science and SciFinder databases and for the different area of interest: i) area 1: Data on chemical identification and characterisation of NOCs analysed in food products; ii) area 2: Data on sources and occurrence in food, including human milk; iii) area 3: Data on toxicokinetics (absorption, distribution, metabolism, excretion) in experimental animals and humans and from *in vitro* studies; area 4: Data on toxicity in experimental animals; area 5: Data on *in silico*, *in vitro* and *in vivo* genotoxicity and mode of action; and area 6: Data on observations in humans (including epidemiological studies, case reports, biomarkers of exposure). Inclusion and exclusion criteria were thoroughly defined in order to foresee the selection of relevance. In addition, data reporting methodology and summary tables were defined and agreed with EFSA.

### *Objective 2*

The extensive literature search (ELS) was carried out using the protocol devised in Objective 1. Three different databases were interrogated, namely, PubMed, Web of Science and SciFinder. In parallel, a literature search specific for grey documents from regulatory agencies and other authorities was also performed. PubMed, Web of Science, Scifinder and the grey literature allowed to identify 8398, 12114, 4009 and 5 redundant documents, respectively. The removal of references that appeared simultaneously to more than one database allowed to obtain a final list of 18592 non-redundant references.

### *Objective 3*

A screening of titles and abstracts for relevance to the risk assessment was performed keeping into account inclusion and exclusion criteria. The experts of the team analysed references divided by area of interest and full-text examination was performed only in case of doubts or where a missing consensus about the relevance was present among experts. For relevant studies high-level information that could be clearly identified were extracted following the data reporting methodology proposed and agreed with EFSA divided by area of interest. The relevance analysis allowed to retrieve the following relevant documents: i) Area 1 176 documents; ii) Area 2 234 documents; Area 3 148 documents; Area 4 143 documents; Area 5 726 documents; Area 6 333 documents. A total number of 1494 non-redundant relevant documents were found. Files compatible with EndNote (RIS file) with the relevant literature were exported and provided as well as tables divided by area with annotated high-level information. In addition, a summary table reporting included/excluded selection with the reason of exclusion was provided.

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## 1. Introduction

### 1.1. Background and Terms of Reference as provided by the requestor

This contract was awarded by EFSA to a consortium with Innovamol Consulting Srl in the lead:

Contractor: Innovamol Consulting Srl

Members of the consortium are:

- Innovamol Consulting Srl, Modena, Italy
- University of Bologna, Bologna, Italy
- University of Foggia, Foggia, Italy

Contract title: Extensive literature search on *N*-nitroso compounds in food <sup>(1)</sup>

Contract number: NP/EFSA/BIOCONTAM/2020/07

### 1.2. Background as provided by EFSA

The Unit on Biological Hazards and Contaminants (BIOCONTAM Unit) supports the Panel on Contaminants in the Food Chain (CONTAM Panel), which provides scientific advice on contaminants in the food chain and undesirable substances such as natural toxicants, mycotoxins and residues of unauthorised substances.

EFSA has received a new mandate from the European Commission for a scientific opinion on the risks to human health related to the presence of *N*-nitrosamines in food. This mandate has been allocated to the CONTAM Panel and a Working Group will be established to develop this opinion. The CONTAM Panel considered to extend this evaluation to *N*-nitroso compounds in food in general.

To support the preparatory work for the hazard identification and characterisation steps in the human health risk assessment, EFSA wishes to outsource an Extensive Literature Search (ELS) as well as the selection of relevant studies by screening of title and abstract related to the chemical identity and characterisation, sources and occurrence in food, toxicokinetics and toxicity of *N*-nitroso compounds (NOCs) in food. NOCs are chemical compounds bearing a nitroso group ( $-N=O$ ) attached to a nitrogen atom ( $N-N=O$ ).

The NOCs can be divided into two classes: *N*-nitrosamines and *N*-nitrosamides and related compounds. *N*-nitrosamines are *N*-nitroso derivatives of secondary amines containing dialkyl, alkylaryl and diaryl substituents (Figure 1A); *N*-nitrosamides and its related compounds are *N*-nitroso derivatives of ureas, amides, carbamates, guanidines, and similar compounds.

### 1.3. Objectives as provided by EFSA

The objectives of the contract resulting from this procurement procedure are as follows:

- Objective 1 (pursued in Task 1): To develop tailored search strategies to retrieve studies (including reviews and grey literature) pertinent to the risk assessment and characterisation of *N*-nitroso compounds (NOCs) found in food products in the following areas:
  - Area 1: Data on chemical identification and characterisation of NOCs found in food products.
  - Area 2: Data on sources and occurrence in food, including human milk.
  - Area 3: Data on toxicokinetics (absorption, distribution, metabolism, excretion) in experimental animals and humans and from *in vitro* studies.

<sup>1</sup> Extensive literature search on nitrosocompounds in food [Internet]. 2020. Available from: <https://www.efsa.europa.eu/en/consultations/call/extensive-literature-search-nitrosocompounds-food>

- Area 4: Data on toxicity in experimental animals.
- Area 5: Data on *in silico*, *in vitro* and *in vivo* genotoxicity and mode of action.
- Area 6: Data on observations in humans (including epidemiological studies, case reports, biomarkers of exposure)

The search strategies proposed by the contractor will be discussed and agreed with EFSA before proceeding with the actual searches.

- Objective 2 (pursued in Task 2): To carry out the extensive literature search (ELS) using the tailored search strings developed by the contractor and agreed by EFSA for identifying relevant studies.
- Objective 3 (pursued in Task 3): To screen the titles and abstracts for relevance to the risk assessment. To prepare a file compatible with EndNote (RIS file) with the relevant literature and a table summarising the relevant studies. These should contain the studies considered as relevant applying the eligibility criteria (for inclusion/exclusion of studies) that have to be developed by the contractor. The eligibility criteria proposed by the contractor will be discussed and agreed with EFSA before proceeding with the actual screening of titles and abstracts. The file compatible with EndNote (RIS file) and table should be organised by group of compounds and by area.

## 2. Data and Methodologies

### 2.1. Objective 1

#### 2.1.1. Preparation of literature search

The protocol for the developed of tailored search included the definition of the following points:

- Definition of Boolean searches. All the database and data sources were able to support automated queries that were managed as specified below with query syntax suitable to obtain output files. Searches were performed with Boolean queries, in particular by using the Boolean operators "AND" and "OR" and parenthesis combinations involving NOCs compounds keywords or chemical substructures and keywords that have been agreed with EFSA during the preparatory meeting. The Boolean operators "NOT" was not used to avoid automatic rejection of potentially relevant documents.
- Keywords and query syntax for PubMed. The selection of keywords and the query syntax were customized and agreed with EFSA by keeping into account different area of interest: i) area 1: Data on chemical identification and characterisation of NOCs found in food products; ii) area 2: Data on sources and occurrence in food, including human milk, iii) area 3: Data on toxicokinetics (absorption, distribution, metabolism, excretion) in experimental animals and humans and from *in vitro* studies, area 4: Data on toxicity in experimental animals, area 5: Data on *in silico*, *in vitro* and *in vivo* genotoxicity and mode of action and area 6: Data on observations in humans (including epidemiological studies, case reports, biomarkers of exposure).
- Keywords and query syntax for Web of Science. Even in this case, the selection of keywords and the query syntax were customized keeping into account different area of interest.
- Keywords and query syntax for SciFinder. For this search queries required a different strategy that is dictated by the different search engine capability of the database itself and the information related to chemical substances. After different test queries performed with Scifinder we devised a strategy composed by 9 steps that will be presented in the result section.
- Definition of searches for other database and grey literature.
- Definition of inclusion criteria.

- Definition of exclusion criteria.
- Definition of reporting methodology and summary tables.

All the above-mentioned points were agreed with EFSA before proceeding to actual searches.

### 2.1.2. Database

The database used for this project were PubMed, Web of Science and SciFinder and, in the case of the grey literature direct access to websites of agencies and authorities in EU and outside EU was performed. Because of the intrinsic differences in each website tailored searches were applied for each source. Queries and date of searches are reported in the result section.

### 2.1.3. Software

Mendeley V.1.19.08<sup>2</sup> was used to manage references and remove duplicated. Microsoft Access build 13127.20313 was used to collect additional information from annotated studies and export tables. Microsoft Excel and Microsoft Word were used to report data in text and table format. JabRef V. 5.2 was used to check RIS files and data integrity. Raw results were collected by exporting references from Pubmed, Scifinder and Web of Science in txt, CSV, BIB or RIS format.

## 2.2. Objective 2

The extensive literature search (ELS) was carried out using the protocol devised in Objective 1. Three different databases were interrogated, namely, PubMed, Web of Science and SciFinder as specified above. In parallel, a literature search specific for grey documents from regulatory agencies and other authorities was also performed. PubMed, Web of Science, Scifinder were collected in the reference manager software as a repository.

The repository of data was post-processed with the software functionalities in order to:

- i) merge data from the different searches,
- ii) remove duplicates,
- iii) perform integrity check of each entry and,
- iv) correct reference citations.

Tags were implemented in Mendeley reference manager in order to process articles coming from different searches and areas. Query syntax, date of performance of searches and hits obtained are reported in the result section. The result of this post-processing returned a non-redundant and complete list of entries directly linked NOC and the various aspects of the tender specifications.

## 2.3. Objective 3

A screening of titles and abstracts for relevance to the risk assessment was performed keeping into account inclusion and exclusion criteria. The experts of the team analysed references divided by area of interest and full-text examination was performed only in case of doubts or where a missing consensus about the relevance was present among experts.

The work was organized in a way that at two experts with different expertise (i.e. toxicology, epidemiology or food occurrence/analysis) could review independently documents, especially in case of borderline documents. A decision on the relevance was made for each record/document if at least one expert judged it relevant as respect to the specific questions of the tender (i.e. inclusion and exclusion criteria). For each document, the following actions were performed:

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<sup>2</sup> Mendeley Desktop [Internet]. Available from: <https://www.mendeley.com/>

- The relevance of each document will be evaluated by checking if relevant keywords describe a real scientific relationship between NOCs and food effects/safety/toxicology.
- The relevance of each document will be evaluated against inclusion criteria.
- The relevance of each document will be evaluated against exclusion criteria.

In many cases, articles were judged clearly irrelevant and excluded without further action. In many other cases the only analysis of the title and abstract was sufficient to judge the article relevant. In this case the appropriate tag of relevance was applied to the record in our internal database and no further action was performed at this stage.

Full-text examination was performed only in case of doubts or where a missing consensus about the relevance was present among experts. In most of the cases the full-text examination allowed to judge documents to be mainly irrelevant, but it is worth to emphasize that in case of borderline documents a conservative approach was used to ensure the completeness and the document were marked as relevant.

### 2.3.1. Summary tables reporting

For relevant studies, high-level information that could be clearly identified in the text were extracted following the data reporting methodology proposed and agreed with EFSA divided by area of interest. High level information data included:

- title, author, journal, year, abstract
- area of interest (i.e. area 1: data on chemical identification and characterization of NOCs found in food products; area 2: data on sources and occurrence in food, including human milk; area 3: data on toxicokinetics (absorption, distribution, metabolism, excretion) in experimental animals and humans and from *in vitro* studies; area 4: data on toxicity in experimental animals; area 5: data on *in silico*, *in vitro* and *in vivo* genotoxicity and mode of action; area 6: data on observations in humans including epidemiological studies, case reports, biomarkers of exposure)
- study endpoint (e.g. acute, short-term, sub-chronic, chronic), test material, reference substances and test species/animals used
- administration and exposure data (e.g. dose, route of administration, vehicle, duration, and frequency of the treatment)
- effect levels and target systems (e.g. dose descriptors, effect level basis, effect description and additional remarks)
- descriptive results of examinations

These information were gathered in appropriate XLS files divided by area of interest.

### 2.3.2. File reporting

Files that were provided at the end of the project included:

- Files compatible with EndNote (RIS file) PubMed, WoS and Scifinder searches.
- Files compatible with EndNote (RIS file) with all non-redundant references.
- Tables divided by area with annotated high-level information.
- Summary table reporting included/excluded selection with the reason of exclusion.

Results of relevant assessment are reported in the Result section.



### 3. Results

#### 3.1. Objective 1

##### 3.1.1. Keywords and query syntax for PubMed

The construction of this query syntax was dictated by the necessity to include all the aspect of the call but also to avoid unnecessary and unfocused documents that appeared in more general definitions. After different test queries performed with PubMed, and in consultation with EFSA, we obtained the syntax depicted in Table 1.

Because documents retrieved from PubMed and Web of Science largely exceeded the capability to perform subsequent tasks, a specific set of documents was obtained from specific searches as depicted in Table 1 and Table 2 below. These searches combined non-redundant results and filtering by reviews. Reviews were identified using though the "review" filter when performing the searches in the PubMed and WoS. The outcome of this filtering allowed to collect reviews that are provided as a deliverable without extraction of high-level information, as agreed with EFSA.

**Table 1:** Keywords for PubMed searches.

N°	Concepts	Syntax/queries	Note
1	<b>Area 1 - Data on chemical identification and characterization of NOCs found in food products, including processing conditions</b>	N-nitroso*[tiab] AND (identification[tiab] OR characterization[tiab] OR analysis[tiab] OR quantification[tiab] OR detection[tiab] OR determination[tiab] OR "Mass spectrometry"[tiab] OR chromatography[tiab] OR spectroscopy*[tiab] OR determin*[tiab] OR method*[tiab] OR form*[tiab] OR reaction*[tiab] OR "GC-MS"[tiab] OR "GC-FID"[tiab] OR "HPLC"[tiab] OR "LC-MS"[tiab] OR "LC-UV"[tiab] OR "NMR"[tiab] OR "thermal energy analyser"[tiab]) AND (food[tiab] OR food[mesh] OR "human milk"[tiab] OR breastfeeding[tiab] OR "breast feeding"[tiab] OR bacon[tiab] OR beer[tiab] OR beverage*[tiab] OR brisket[tiab] OR cheese[tiab] OR chorizo[tiab] OR cured[tiab] OR dairy[tiab] OR fat[tiab] OR fish[tiab] OR ham[tiab] OR herring[tiab] OR jambon[tiab] OR meat[tiab] OR milk[tiab] OR offal[tiab] OR oil[tiab] OR pâtés[tiab] OR potatoes[tiab] OR poultry[tiab] OR salami[tiab] OR saucisson*[tiab] OR sausage[tiab] OR soup*[tiab] OR soy[tiab] OR spice*[tiab] OR sprat[tiab] OR tea[tiab] OR terrine[tiab] OR vegetable*[tiab] OR whey[tiab] OR "cold cut"[tiab] OR "infant formulae"[tiab] OR "game animals"[tiab] OR "smoked food"[tiab] OR Maillard[tiab])	filter activated: english
2	<b>Area 2 - Data on sources and occurrence in food, including human milk</b>	N-nitroso*[tiab] AND (sources[tiab] OR occurrence[tiab] OR exposure[tiab] OR formation[tiab] OR generation[tiab] OR list[tiab] OR database[tiab]) AND (food[tiab] OR food[mesh] OR "human milk"[tiab] OR breastfeeding[tiab] OR "breast feeding"[tiab] OR bacon[tiab] OR beer[tiab] OR beverage*[tiab] OR brisket[tiab] OR cheese[tiab] OR chorizo[tiab] OR cured[tiab] OR dairy[tiab] OR fat[tiab] OR fish[tiab] OR ham[tiab] OR herring[tiab] OR jambon[tiab] OR meat[tiab] OR milk[tiab] OR offal[tiab] OR oil[tiab] OR pâtés[tiab] OR potatoes[tiab] OR poultry[tiab] OR salami[tiab] OR saucisson*[tiab] OR sausage[tiab] OR soup*[tiab] OR soy[tiab] OR spice*[tiab] OR sprat[tiab] OR tea[tiab] OR terrine[tiab] OR vegetable*[tiab] OR whey[tiab] OR "cold cut"[tiab] OR "infant formulae"[tiab] OR "game animals"[tiab] OR "smoked food"[tiab] OR Maillard[tiab])	filter activated: english
3	<b>Area 3 - Data on toxicokinetics (absorption, distribution, metabolism, excretion) in experimental animals and humans and from <i>in vitro</i> studies.</b>  <b>For nitrosamines</b>  <b>Animal studies</b>	((nitroso[tiab] OR nitrosam*[tiab] OR nitrosoam*[tiab]) AND (ethyl*[tiab] OR methyl*[tiab] OR propyl*[tiab] OR morpholin*[tiab] OR ethylmethyl[tiab] OR methylethyl[tiab] OR pyrrol*[tiab] OR methylvinyl*[tiab] OR piperidin*[tiab] OR butyl*[tiab] OR phenyl*[tiab] OR proline[tiab] OR sarcosine[tiab] OR methylaniline[tiab] OR pipecolic[tiab] OR benzyl[tiab] OR thiazolidine[tiab] OR oxazolidine[tiab])) AND ( <i>in vitro</i> [tiab] OR <i>in vivo</i> [tiab] OR absor*[tiab] OR distributio*[tiab] OR tissue*[tiab] OR metaboli*[tiab] OR excret*[tiab] OR kinetic*[tiab] OR toxicokinetic*[tiab] OR pharmacokinetic*[tiab] OR degrad*[tiab] OR biotrans*[tiab] OR eliminat*[tiab] OR residue[tiab]) AND (mink[tiab] OR cat[tiab] OR dog[tiab] OR rat[tiab] OR mouse[tiab] OR mice[tiab] OR rabbit*[tiab] OR pig[tiab] OR hamster*[tiab] OR primate*[tiab] OR monkey[tiab] OR animal[tiab])	filter activated: english

<p><b>4</b></p>	<p><b>Area 3 - Data on toxicokinetics (absorption, distribution, metabolism, excretion) in experimental animals and humans and from <i>in vitro</i> studies.</b></p> <p><b>For all NOCs</b></p> <p><b>Animal studies</b></p>	<p>(nitros*[tiab]) AND (<i>in vitro</i>[tiab] OR <i>in vivo</i>[tiab] OR absor*[tiab] OR distributio*[tiab] OR tissue*[tiab] OR metaboli*[tiab] OR excret*[tiab] OR kinetic*[tiab] OR toxicokinetic*[tiab] OR pharmacokinetic*[tiab] OR degrad*[tiab] OR biotrans*[tiab] OR eliminat*[tiab] OR residue[tiab]) AND (mink[tiab] OR cat[tiab] OR dog[tiab] OR rat[tiab] OR mouse[tiab] OR mice[tiab] OR rabbit*[tiab] OR pig[tiab] OR hamster*[tiab] OR primate*[tiab] OR monkey[tiab] OR animal[tiab])</p>	<p>filter activated: english, review</p>
<p><b>5</b></p>	<p><b>Area 3 - Data on toxicokinetics (absorption, distribution, metabolism, excretion) in experimental animals and humans and from <i>in vitro</i> studies.</b></p> <p><b>For nitrosamines</b></p> <p><b>Human studies</b></p>	<p>((nitroso[tiab] OR nitrosam*[tiab] OR nitrosoam*[tiab]) AND (ethyl*[tiab] or methyl*[tiab] OR propyl*[tiab] OR morpholin*[tiab] OR ethylmethyl[tiab] OR methylethyl[tiab] OR pyrrol*[tiab] OR methylvinyl*[tiab] OR piperidin*[tiab] OR butyl*[tiab] OR phenyl*[tiab] OR proline[tiab] OR sarcosine[tiab] OR methylaniline[tiab] OR pipercolic[tiab] OR benzyl[tiab] OR thiazolidine[tiab] OR oxazolidine[tiab])) AND (<i>in vitro</i>[tiab] OR <i>in vivo</i>[tiab] OR absor*[tiab] OR distributio*[tiab] OR tissue*[tiab] OR metaboli*[tiab] OR excret*[tiab] OR kinetic*[tiab] OR toxicokinetic*[tiab] OR pharmacokinetic*[tiab] OR degrad*[tiab] OR biotrans*[tiab] OR eliminat*[tiab] OR residue[tiab]) AND (human[tiab])</p>	<p>filter activated: english</p>
<p><b>6</b></p>	<p><b>Area 3 - Data on toxicokinetics (absorption, distribution, metabolism, excretion) in experimental animals and humans and from <i>in vitro</i> studies.</b></p> <p><b>For all NOCs</b></p> <p><b>Human studies</b></p>	<p>(nitros*[tiab]) AND (<i>in vitro</i>[tiab] OR <i>in vivo</i>[tiab] OR absor*[tiab] OR distributio*[tiab] OR tissue*[tiab] OR metaboli*[tiab] OR excret*[tiab] OR kinetic*[tiab] OR toxicokinetic*[tiab] OR pharmacokinetic*[tiab] OR degrad*[tiab] OR biotrans*[tiab] OR eliminat*[tiab] OR residue[tiab]) AND (human[tiab])</p>	<p>filter activated: english, review</p>
<p><b>7</b></p>	<p><b>Area 4 - Data on toxicity in experimental animals</b></p> <p><b>For nitrosamines</b></p>	<p>((nitroso[tiab] OR nitrosam*[tiab] OR nitrosoam*[tiab]) AND (ethyl*[tiab] or methyl*[tiab] OR propyl*[tiab] OR morpholin*[tiab] OR ethylmethyl[tiab] OR methylethyl[tiab] OR pyrrol*[tiab] OR methylvinyl*[tiab] OR piperidin*[tiab] OR butyl*[tiab] OR phenyl*[tiab] OR proline[tiab] OR sarcosine[tiab] OR methylaniline[tiab] OR pipercolic[tiab] OR benzyl[tiab] OR thiazolidine[tiab] OR oxazolidine[tiab])) AND (<i>in vivo</i>[tiab] OR acute[tiab] OR chronic*[tiab] OR toxi*[tiab] OR cancer*[tiab] OR carcino*[tiab] OR tumor*[tiab] OR tumour*[tiab] OR organ*[tiab] OR tissue*[tiab] OR immun*[tiab] OR neuro*[tiab] OR developmental[tiab] OR teratogen*[tiab] OR repro*[tiab] OR liver[tiab] OR kidney*[tiab] OR brain*[tiab] OR lung*[tiab] OR gastr*[tiab] OR intestin*[tiab] OR nasal*[tiab] OR oesophag*[tiab] OR esophag*[tiab] OR bladder[tiab] OR gland*[tiab] OR stomach*[tiab] OR pancrea*[tiab] OR mucosa*[tiab] OR sarcom*[tiab] OR toxic*[tiab] OR survi*[tiab] OR morta*[tiab] OR lethal*[tiab] OR adverse[tiab] OR acute[tiab] OR diet[tiab] OR dose[tiab] OR gavage[tiab] OR irrit*[tiab] OR sensitiz*[tiab]) AND (mink[tiab] OR cat[tiab] OR dog[tiab] OR rat[tiab] OR mouse[tiab] OR mice[tiab] OR rabbit*[tiab] OR pig[tiab] OR hamster*[tiab] OR primate*[tiab] OR monkey[tiab] OR animal[tiab])</p>	<p>filter activated: english</p>

8	<b>Area 4 - Data on toxicity in experimental animals</b>  <b>For all NOCs</b>	(nitros*[tiab]) AND ( <i>in vivo</i> [tiab] OR acute[tiab] OR chronic*[tiab] OR toxic*[tiab] OR cancer*[tiab] OR carcino*[tiab] OR tumor*[tiab] OR tumour*[tiab] OR organ*[tiab] OR tissue*[tiab] OR immun*[tiab] OR neuro*[tiab] OR developmental[tiab] OR teratogen*[tiab] OR repro*[tiab] OR liver[tiab] OR kidney*[tiab] OR brain*[tiab] OR lung*[tiab] OR gastr*[tiab] OR intestin*[tiab] OR nasal*[tiab] OR oesophag*[tiab] OR esophag*[tiab] OR bladder[tiab] OR gland*[tiab] OR stomac*[tiab] OR pancrea*[tiab] OR mucosa*[tiab] OR sarcom*[tiab] OR toxic*[tiab] OR survi*[tiab] OR morta*[tiab] OR lethal*[tiab] OR adverse[tiab] OR acute[tiab] OR diet[tiab] OR dose[tiab] OR gavage[tiab] OR irrit*[tiab] OR sensit*[tiab]) AND (mink[tiab] OR cat[tiab] OR dog[tiab] OR rat[tiab] OR mouse[tiab] OR mice[tiab] OR rabbit*[tiab] OR pig[tiab] OR hamster*[tiab] OR primate*[tiab] OR monkey[tiab] OR animal[tiab])	filter activated: english, review
9	<b>Area 5 - Data on <i>in silico</i>, <i>in vitro</i> and <i>in vivo</i> genotoxicity and mode of action</b>  <b>For nitrosamines</b>  <b>Genotoxicity</b>	((nitroso[tiab] OR nitrosam*[tiab] OR nitrosoam*[tiab]) AND (ethyl*[tiab] or methyl*[tiab] OR propyl*[tiab] OR morpholin*[tiab] OR ethylmethyl[tiab] OR methylethyl[tiab] OR pyrrol*[tiab] OR methylvinyl*[tiab] OR piperidin*[tiab] OR butyl*[tiab] OR phenyl*[tiab] OR proline[tiab] OR sarcosine[tiab] OR methylaniline[tiab] OR pipecolic[tiab] OR benzyl[tiab] OR thiazolidine[tiab] OR oxazolidine[tiab])) AND ( <i>in vitro</i> [tiab] OR <i>in vivo</i> [tiab] OR cytotox*[tiab] OR genotox*[tiab] OR muta*[tiab] OR damage[tiab] OR repair[tiab] OR clastogen*[tiab] OR aneugen*[tiab] OR chromosom*[tiab] OR carcinogen*[tiab] OR tumour[tiab] OR interaction[tiab] OR DNA binding[tiab] OR micronucle*[tiab])	filter activated: english
10	<b>Area 5 - Data on <i>in silico</i>, <i>in vitro</i> and <i>in vivo</i> genotoxicity and mode of action</b>  <b>For all NOCs</b>  <b>Genotoxicity</b>	(nitros*[tiab]) AND ( <i>in vitro</i> [tiab] OR <i>in vivo</i> [tiab] OR cytotox*[tiab] OR genotox*[tiab] OR muta*[tiab] OR damage[tiab] OR repair[tiab] OR clastogen*[tiab] OR aneugen*[tiab] OR chromosom*[tiab] OR carcinogen*[tiab] OR tumour[tiab] OR interaction[tiab] OR DNA binding[tiab] OR micronucle*[tiab])	filter activated: english, review
11	<b>Area 5 - Data on <i>in silico</i>, <i>in vitro</i> and <i>in vivo</i> genotoxicity and mode of action</b>  <b>For nitrosamines</b>  <b>Mode of action</b>	((nitroso[tiab] OR nitrosam*[tiab] OR nitrosoam*[tiab]) AND (ethyl*[tiab] or methyl*[tiab] OR propyl*[tiab] OR morpholin*[tiab] OR ethylmethyl[tiab] OR methylethyl[tiab] OR pyrrol*[tiab] OR methylvinyl*[tiab] OR piperidin*[tiab] OR butyl*[tiab] OR phenyl*[tiab] OR proline[tiab] OR sarcosine[tiab] OR methylaniline[tiab] OR pipecolic[tiab] OR benzyl[tiab] OR thiazolidine[tiab] OR oxazolidine[tiab])) AND ( <i>in vitro</i> [tiab] OR <i>in vivo</i> [tiab] OR mode of action[tiab] OR mechanism[tiab] OR Adverse outcome pathway[tiab] OR mechanism*[tiab] OR apoptosis[tiab] OR oxidative damage[tiab] OR oxidative stress[tiab])	filter activated: english
12	<b>Area 5 - Data on <i>in silico</i>, <i>in vitro</i> and <i>in vivo</i> genotoxicity and mode of action</b>  <b>For all NOCs</b>  <b>Mode of action</b>	(nitros*[tiab]) AND ( <i>in vitro</i> [tiab] OR <i>in vivo</i> [tiab] OR mode of action[tiab] OR mechanism[tiab] OR Adverse outcome pathway[tiab] OR mechanism*[tiab] OR apoptosis[tiab] OR oxidative damage[tiab] OR oxidative stress[tiab])	filter activated: english, review
13	<b>Area 6 - Data on observations in humans (including epidemiological studies, case reports, biomarkers of exposure)</b>	N-nitros*[tiab] AND ((epidemi*[tiab] OR biomark*[tiab] OR exposure*[tiab] OR case-control[tiab] OR poison*[tiab] OR cohort*[tiab] OR cross-sectional[tiab] OR case-control[tiab] OR cancer*[tiab] OR health[tiab] OR disease[tiab] OR medic*[tiab] OR "general population"[tiab] OR biomonit*[tiab] OR (level*[tiab] AND (serum[tiab] OR urine[tiab] OR breast-milk[tiab]))) AND (humans[tiab] OR subject*[tiab] OR population*[tiab]))	filter activated: english

### 3.1.2. Web of Science.

Web of Science searches are depicted in Table 2 and are defined similarly to PubMed queries.

**Table 2:** Keywords for Web of Science searches.

N°	Concepts	Syntax/queries	Note
1	<b>Area 1 - Data on chemical identification and characterization of NOCs found in food products, including processing conditions</b>	TOPIC: ((N-nitros*) AND (identification OR characterization OR analysis OR quantification OR detection OR determination OR "Mass spectrometry" OR chromatography OR spectroscop* OR determin* OR method* OR form* OR reaction* OR "GC-MS" OR "GC-FID" OR "HPLC" OR "LC-MS" OR "LC-UV" OR "NMR" OR "thermal energy analyser")) AND (food OR "human milk" OR breastfeeding OR "breast feeding" OR bacon OR beer OR beverage* OR brisket OR cheese OR chorizo OR cured OR dairy OR fat OR fish OR ham OR herring OR jambon OR meat OR milk OR offal OR oil OR pâtés OR potatoes OR poultry OR salami OR saucisson* OR sausage OR soup* OR soy OR spice* OR sprat OR tea OR terrine OR vegetable* OR whey OR "cold cut" OR "infant formulae" OR "game animals" OR "smoked food" OR Maillard))	Database: Core Collection filter activated: english
2	<b>Area 2 - Data on sources and occurrence in food, including human milk</b>	TOPIC: ((N-nitros*) AND (sources OR occurrence OR exposure OR formation OR generation OR list OR database) AND (food OR "human milk" OR breastfeeding OR "breast feeding" OR bacon OR beer OR beverage* OR brisket OR cheese OR chorizo OR cured OR dairy OR fat OR fish OR ham OR herring OR jambon OR meat OR milk OR offal OR oil OR pâtés OR potatoes OR poultry OR salami OR saucisson* OR sausage OR soup* OR soy OR spice* OR sprat OR tea OR terrine OR vegetable* OR whey OR "cold cut" OR "infant formulae" OR "game animals" OR "smoked food" OR Maillard))	Database: Core Collection filter activated: english
3	<b>Area 3 - Data on toxicokinetics (absorption, distribution, metabolism, excretion) in experimental animals and humans and from <i>in vitro</i> studies.</b>  <b>For nitrosamines</b>  <b>Animal studies</b>	TOPIC: (((nitroso OR nitrosam* OR nitrosoam*) AND (ethyl* OR methyl* OR propyl* OR morpholin* OR ethylmethyl OR methylethyl OR pyrrol* OR methylvinyl* OR piperidin* OR butyl* OR phenyl* OR proline OR sarcosine OR methylaniline OR pipercolic OR benzyl OR thiazolidine OR oxazolidine)) AND ("in vitro" OR "in vivo" OR absor* OR distributio* OR tissue* OR metaboli* OR excret* OR kinetic* OR toxicokinetic* OR pharmacokinetic* OR degrad* OR biotrans* OR eliminat* OR residue) AND (mink OR cat OR dog OR rat OR mouse OR mice OR rabbit* OR pig OR hamster* OR primate* OR monkey OR animal))	Database: Core Collection filter activated: english
4	<b>Area 3 - Data on toxicokinetics (absorption, distribution, metabolism, excretion) in experimental animals and humans and from <i>in vitro</i> studies.</b>  <b>For all NOCs</b>  <b>Animal studies</b>	TOPIC: ((nitros*) AND ("in vitro" OR "in vivo" OR absor* OR distributio* OR tissue* OR metaboli* OR excret* OR kinetic* OR toxicokinetic* OR pharmacokinetic* OR degrad* OR biotrans* OR eliminat* OR residue) AND (mink OR cat OR dog OR rat OR mouse OR mice OR rabbit* OR pig OR hamster* OR primate* OR monkey OR animal))	Database: Core Collection filter activated: English, review

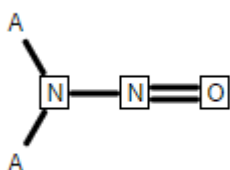
5	<p><b>Area 3 - Data on toxicokinetics (absorption, distribution, metabolism, excretion) in experimental animals and humans and from <i>in vitro</i> studies.</b></p> <p><b>For nitrosamines</b></p> <p><b>Human studies</b></p>	<p>TOPIC: (((nitroso OR nitrosam* OR nitrosoam*) AND (ethyl* or methyl* OR propyl* OR morpholin* OR ethylmethyl OR methylethyl OR pyrrol* OR methylvinyl* OR piperidin* OR butyl* OR phenyl* OR proline OR sarcosine OR methylaniline OR pipercolic OR benzyl OR thiazolidine OR oxazolidine)) AND ("in vitro" OR "in vivo" OR absor* OR distributio* OR tissue* OR metaboli* OR excret* OR kinetic* OR toxicokinetic* OR pharmacokinetic* OR degrad* OR biotrans* OR eliminat* OR residue) AND (human))</p>	<p>Database: Core Collection filter activated: english</p>
6	<p><b>Area 3 - Data on toxicokinetics (absorption, distribution, metabolism, excretion) in experimental animals and humans and from <i>in vitro</i> studies.</b></p> <p><b>For all NOCs</b></p> <p><b>Human studies</b></p>	<p>TOPIC: ((nitros*) AND (<i>in vitro</i> OR <i>in vivo</i> OR absor* OR distributio* OR tissue* OR metaboli* OR excret* OR kinetic* OR toxicokinetic* OR pharmacokinetic* OR degrad* OR biotrans* OR eliminat* OR residue) AND (human))</p>	<p>Database: Core Collection filter activated: English, review</p>
7	<p><b>Area 4 - Data on toxicity in experimental animals</b></p> <p><b>For nitrosamines</b></p>	<p>TOPIC: (((nitroso OR nitrosam* OR nitrosoam*) AND (ethyl* or methyl* OR propyl* OR morpholin* OR ethylmethyl OR methylethyl OR pyrrol* OR methylvinyl* OR piperidin* OR butyl* OR phenyl* OR proline OR sarcosine OR methylaniline OR pipercolic OR benzyl OR thiazolidine OR oxazolidine)) AND ("in vivo" OR acute OR chronic* OR toxi* OR cancer* OR carcino* OR tumor* OR tumour* OR organ* OR tissue* OR immun* OR neuro* OR developmental OR teratogen* OR repro* OR liver OR kidney* OR brain* OR lung* OR gastr* OR intestin* OR nasal* OR oesophag* OR esophag* OR bladder OR gland* OR stomach* OR pancrea* OR mucosa* OR sarcom* OR toxic* OR survi* OR morta* OR lethal* OR adverse OR acute OR diet OR dose OR gavage OR irrit* OR sensitis*) AND (mink OR cat OR dog OR rat OR mouse OR mice OR rabbit* OR pig OR hamster* OR primate* OR monkey OR animal))</p>	<p>Database: Core Collection filter activated: english</p>
8	<p><b>Area 4 - Data on toxicity in experimental animals</b></p> <p><b>For all NOCs</b></p>	<p>TOPIC: ((nitros*) AND ("in vivo" OR acute OR chronic* OR toxi* OR cancer* OR carcino* OR tumor* OR tumour* OR organ* OR tissue* OR immun* OR neuro* OR developmental OR teratogen* OR repro* OR liver OR kidney* OR brain* OR lung* OR gastr* OR intestin* OR nasal* OR oesophag* OR esophag* OR bladder OR gland* OR stomach* OR pancrea* OR mucosa* OR sarcom* OR toxic* OR survi* OR morta* OR lethal* OR adverse OR acute OR diet OR dose OR gavage OR irrit* OR sensitis*) AND (mink OR cat OR dog OR rat OR mouse OR mice OR rabbit* OR pig OR hamster* OR primate* OR monkey OR animal))</p>	<p>Database: Core Collection filter activated: English, review</p>
9	<p><b>Area 5 - Data on <i>in silico</i>, <i>in vitro</i> and <i>in vivo</i> genotoxicity and mode of action</b></p> <p><b>For nitrosamines</b></p> <p><b>Genotoxicity</b></p>	<p>TOPIC: (((nitroso OR nitrosam* OR nitrosoam*) AND (ethyl* or methyl* OR propyl* OR morpholin* OR ethylmethyl OR methylethyl OR pyrrol* OR methylvinyl* OR piperidin* OR butyl* OR phenyl* OR proline OR sarcosine OR methylaniline OR pipercolic OR benzyl OR thiazolidine OR oxazolidine)) AND ("in vitro" OR "in vivo" OR cytotox* OR genotox* OR muta* OR damage OR repair OR clastogen* OR aneugen* OR chromosom* OR carcinogen* OR tumour OR interaction OR "DNA binding" OR micronucle*))</p>	<p>Database: Core Collection filter activated: english</p>
10	<p><b>Area 5 - Data on <i>in silico</i>, <i>in vitro</i> and <i>in vivo</i> genotoxicity</b></p>	<p>TOPIC: ((nitros*) AND ("in vitro" OR "in vivo" OR cytotox* OR genotox* OR muta* OR damage OR repair OR clastogen* OR aneugen* OR chromosom* OR carcinogen* OR tumour OR interaction OR "DNA binding" OR micronucle*))</p>	<p>Database: Core Collection</p>

	<b>and mode of action</b>  <b>For all NOCs</b>  <b>Genotoxicity</b>		filter activated: English, review
11	<b>Area 5 - Data on <i>in silico</i>, <i>in vitro</i> and <i>in vivo</i> genotoxicity and mode of action</b>  <b>For nitrosamines</b>  <b>Mode of action</b>	TOPIC: (((nitroso OR nitrosam* OR nitrosoam*) AND (ethyl* OR methyl* OR propyl* OR morpholin* OR ethylmethyl OR methylethyl OR pyrrol* OR methylvinyl* OR piperidin* OR butyl* OR phenyl* OR proline OR sarcosine OR methylaniline OR pipercolic OR benzyl OR thiazolidine OR oxazolidine)) AND ("in vitro" OR "in vivo" OR "mode of action" OR mechanism OR "Adverse outcome pathway" OR mechanism* OR apoptosis OR "oxidative damage" OR "oxidative stress"))	Database: Core Collection filter activated: english
12	<b>Area 5 - Data on <i>in silico</i>, <i>in vitro</i> and <i>in vivo</i> genotoxicity and mode of action</b>  <b>For all NOCs</b>  <b>Mode of action</b>	TOPIC: ((nitros*) AND ("in vitro" OR "in vivo" OR "mode of action" OR mechanism OR "Adverse outcome pathway" OR mechanism* OR apoptosis OR "oxidative damage" OR "oxidative stress"))	Database: Core Collection filter activated: English, review
13	<b>Area 6 - Data on observations in humans (including epidemiological studies, case reports, biomarkers of exposure)</b>	TOPIC: (N-nitros* AND ((epidemi* OR biomark* OR exposure* OR case* OR poison* OR cohort* OR "cross-sectional" OR "case-control" OR cancer* OR health OR disease OR medic* OR "general population" OR biomonit* OR (level* AND (serum OR urine OR "breast milk")))) AND (humans OR subject* OR population*))	Database: Core Collection filter activated: english

### 3.1.3. Scifinder

As anticipated in the methodology section, Scifinder searches required a specific protocol that was devised following the 9 steps below.

1. Chemical substructure search (SSS) with the following structure:



Where A represents any atom except H and squared atoms are locked.

2. Refine substances to retrieve only chemical substances that:
  - a. Have references.
  - b. Are a single component.
  - c. Are in specific substance classes: organics, and others not listed.
  - d. Are in specific types of studies: analytical and biological.
3. Refine substances to exclude chemical substances that:
  - a. Contain isotopes

4. Refine substances to exclude chemical substances that:
  - a. Contain metals
5. Get references from substances limiting results to:
  - a. Adverse effect, including toxicity.
  - b. Analytical studies.
  - c. Biological studies.
6. Refine references to retrieve only studies with the following research topic:
  - a. "food"
7. Refine references to retrieve only studies with the following language:
  - a. English
8. Refine references to retrieve only the following study type:
  - a. Biography
  - b. Book
  - c. Clinical trial
  - d. Commentary
  - e. Conference
  - f. Dissertation
  - g. Editorial
  - h. Historical
  - i. Journal
  - j. Letter
  - k. Report
  - l. Review
9. Removal of duplicate using the SciFinder tool – Final list.

The refinement of reference with the research topic "food" performed at step 6 was necessary to restrict the search to a number to feasible documents for this assignment.

### 3.1.4. Other database

Grey literature searches were performed by accessing websites of agencies and authorities in EU and outside EU and by searching guidances, regulations and scientific opinions. Because of the intrinsic differences in each website, a tailored search was applied for each source. In general, relevant documents were searched by using the internal search engine of the website and different combinations of the search terms as depicted in **Error! Reference source not found.**, including NOS keywords.

### 3.1.5. Inclusion criteria

As a general rule, articles describing occurrence, characterization, or toxicity data in humans and/or animals of NOCs found in food and feed were included as relevant; in particular the areas depicted in Table 3: were considered, as specified in the tender:

**Table 3:** Description of inclusion criteria and numbering used in the present document.

	Description of inclusion criteria
<b>Area 1</b>	Data on chemical identification and characterization of NOCs found in food products, including processing conditions
<b>Area 2</b>	Data on sources and occurrence in food, including human milk

<b>Area 3</b>	Data on toxicokinetics (absorption, distribution, metabolism, excretion) in experimental animals and humans and from <i>in vitro</i> studies
<b>Area 4</b>	Data on toxicity in experimental animals
<b>Area 5</b>	Data on <i>in silico</i> , <i>in vitro</i> and <i>in vivo</i> genotoxicity and mode of action
<b>Area 6</b>	Data on observations in humans (including epidemiological studies, case reports, biomarkers of exposure)

In order to comply with inclusion criteria, each reference documents needed to involve at least one of the areas above. If a document did not involve any of the area above but still described occurrence, characterization, or toxicity data of NOCs found in food it was included exceptionally in the list of relevant studies.

### 3.1.6. Exclusion criteria

To allow accounting in an efficient and unambiguous manner the reasons for excluding a particular document, we devised the scheme depicted in Table 4 where we encoded exclusion criteria, i.e. a codification of the reasons explaining why a specific document needed to be excluded. Exclusion codes were reported in the repository but were not reported as high-level information.

**Table 4:** Exclusion criteria applied to remove irrelevant studies and respective areas involved.

Exclusion code	Area(s) involved	Description of exclusion criteria
<b>EC1</b>	<b>1, 2</b>	NOC used as reagents or chemical building blocks
<b>EC2</b>	<b>1, 2</b>	NOC described in organic chemistry works with the purpose to study new synthetic routes or pathways
<b>EC3</b>	<b>All areas</b>	Documents describing endogenous formation of NOCs
<b>EC4</b>	<b>3, 4</b>	NOC with no direct link to toxicological data in humans or animals
<b>EC5</b>	<b>3, 4, 5</b>	NOC administered with routes other than oral (e.g. gavage, feed, capsules) and intraperitoneal. Excluded: inhalation, dermal, intravenous, subcutaneous, etc.
<b>EC6</b>	<b>3, 5</b>	Standard or well-known genotoxic NOCs used in molecular biology and/or cell biology experiments as tool compounds (e.g. to induce genotoxicity, mutagenicity and/or carcinogenicity)
<b>EC7</b>	<b>All areas</b>	Studies on NOC not analysed or not linked to food (i.e. smoking products and processing, organic solvents, insecticides, disinfectants, environmental carcinogens etc.)
<b>EC8</b>	<b>All areas</b>	Documents (unless reviews) with general speculation, general description or historical description of NOC without link to toxicology, chemical identification or characterization, source data or epidemiological data of NOC compounds
<b>EC9</b>	<b>All areas</b>	NOC that cannot be categorized in Areas 1-6 (unless reviews)

## 3.2. Objective 2

ELS was performed on the requested database with the methodology described in the previous chapter (Objective 1). Individual results of database searches are reported below divided by area of interest.

### 3.2.1. Pubmed searches

PubMed searches were performed on 12/03/2021. Table 5: shows the list of identified documents in PubMed searches. It is worth to notice that searches 4, 6, 8, 10 and 12 were filtered by reviews as reported above. The other searches included original papers that were the object of the relevance assessment and the extraction of high-level information.



**Table 5:** List of identified documents for PubMed searches

Search N°	Concepts	Hits
1	Area 1 - Data on chemical identification and characterization of NOCs found in food products, including processing conditions	1242
2	Area 2 - Data on sources and occurrence in food, including human milk	756
3	Area 3 - Data on toxicokinetics (absorption, distribution, metabolism, excretion) in experimental animals and humans and from <i>in vitro</i> studies. Nitrosamines - Animal studies	1336
4	Area 3 - Data on toxicokinetics (absorption, distribution, metabolism, excretion) in experimental animals and humans and from <i>in vitro</i> studies. NOCs, including non-nitrosamine compounds – Review search - Animal studies	331
5	Area 3 - Data on toxicokinetics (absorption, distribution, metabolism, excretion) in experimental animals and humans and from <i>in vitro</i> studies. Nitrosamines - Human studies	652
6	Area 3 - Data on toxicokinetics (absorption, distribution, metabolism, excretion) in experimental animals and humans and from <i>in vitro</i> studies. NOCs, including non-nitrosamine compounds – Review search - Human studies	367
7	Area 4 - Data on toxicity in experimental animals. Nitrosamines	2262
8	Area 4 - Data on toxicity in experimental animals. NOCs, including non-nitrosamine compounds – Review search	542
9	Area 5 - Data on <i>in silico</i> , <i>in vitro</i> and <i>in vivo</i> genotoxicity and mode of action. Nitrosamines - Genotoxicity	3466
10	Area 5 - Data on <i>in silico</i> , <i>in vitro</i> and <i>in vivo</i> genotoxicity and mode of action. NOCs, including non-nitrosamine compounds – Review search - Genotoxicity	1699
11	Area 5 - Data on <i>in silico</i> , <i>in vitro</i> and <i>in vivo</i> genotoxicity and mode of action. Nitrosamines - Mode of action	1965
12	Area 5 - Data on <i>in silico</i> , <i>in vitro</i> and <i>in vivo</i> genotoxicity and mode of action. NOCs, including non-nitrosamine compounds – Review search - Mode of action	1816
13	Area 6 - Data on observations in humans (including epidemiological studies, case reports, biomarkers of exposure)	978

### 3.2.2. Web of Science searches

Web of Science searches were performed on 12/03/2021. Table 6: shows the list of identified documents in Web of Science searches. As for the case of PubMed searches, it is worth to notice that searches 4, 6, 8, 10 and 12 were filtered by reviews as reported above. The other searches included original papers that were the object of the relevance assessment and the extraction of high-level information.

**Table 6:** List of identified documents for Web of Science searches

Search N°	Concepts	Hits
1	Area 1 - Data on chemical identification and characterization of NOCs found in food products, including processing conditions	1853
2	Area 2 - Data on sources and occurrence in food, including human milk	1208
3	Area 3 - Data on toxicokinetics (absorption, distribution, metabolism, excretion) in experimental animals and humans and from <i>in vitro</i> studies. Nitrosamines - Animal studies	1875
4	Area 3 - Data on toxicokinetics (absorption, distribution, metabolism, excretion) in experimental animals and humans and from <i>in vitro</i> studies. NOCs, including non-nitrosamine compounds – Review search - Animal studies	672
5	Area 3 - Data on toxicokinetics (absorption, distribution, metabolism, excretion) in experimental animals and humans and from <i>in vitro</i> studies. Nitrosamines - Human studies	1044
6	Area 3 - Data on toxicokinetics (absorption, distribution, metabolism, excretion) in experimental animals and humans and from <i>in vitro</i> studies. NOCs, including non-nitrosamine compounds – Review search - Human studies	698

7	Area 4 - Data on toxicity in experimental animals. Nitrosamines	3024
8	Area 4 - Data on toxicity in experimental animals. NOCs, including non-nitrosamine compounds – Review search	1013
9	Area 5 - Data on <i>in silico</i> , <i>in vitro</i> and <i>in vivo</i> genotoxicity and mode of action. Nitrosamines - Genotoxicity	4084
10	Area 5 - Data on <i>in silico</i> , <i>in vitro</i> and <i>in vivo</i> genotoxicity and mode of action. NOCs, including non-nitrosamine compounds – Review search - Genotoxicity	2613
11	Area 5 - Data on <i>in silico</i> , <i>in vitro</i> and <i>in vivo</i> genotoxicity and mode of action. Nitrosamines - Mode of action	2449
12	Area 5 - Data on <i>in silico</i> , <i>in vitro</i> and <i>in vivo</i> genotoxicity and mode of action. NOCs, including non-nitrosamine compounds – Review search - Mode of action	2705
13	Area 6 - Data on observations in humans (including epidemiological studies, case reports, biomarkers of exposure)	3351

### 3.2.3. SciFinder searches

Scifinder searches were performed on 15/03/2021. For Scifinder searches we followed the procedure specified above. In the Table 7: we report the hits of structures and reference that were identified in the various steps. It is worth to emphasize that the refinement performed at Step 6 in terms of concept related to food was necessary to restrict the number of references to a feasible number.

**Table 7:** List of identified documents for Web of Science searches

Step N°	SciFinder steps	Hits
1	Chemical structure with limiters	4753 structures
2	Refine "substructure"	4753 structures
3	Refine "exclude isotope-containing"	4640 structures
4	Refine "exclude metal-containing"	4575 structures
5	Get references	75849 references
6	Refine "food"	5775 references
7	Refine "English"	4934 references
8	Refine document type	4846 references
9	Duplicate removal tool – Final list	4009 references

### 3.2.4. Combined databases

The number of cumulative references identified with ELS are depicted in **Error! Reference source not found.** Several references were simultaneously present in different database and this explains why sum of hits from the three databases plus the grey literature is greater than the total non-redundant references. According to these searches, ELS allowed to obtain 18592 references.

**Table 8:** List of identified documents by combination of all databases

Database	Hits	RIS file
PubMed	8398	pubmed_search_global.ris
Web of Science	12114	WoS_search_global.ris
Scifinder	4009	scifinder_search_global.ris
Grey literature	5	PDF files provided
All references	18592 <sup>a</sup>	All_database_no_dup.ris

<sup>a</sup> Some duplicate articles could not be identified by software at this stage. It is anticipated that these duplicate were removed in Task3 (Objective 3). Thus, the correspondent excel file containing the list of all articles reports 18121 records.

### 3.3. Objective 3

The selection for relevance was performed following the methodology reported in the previous sections. The table below reports the number of relevant documents that were identified divided by area of interest (<https://doi.org/10.5281/zenodo.7128064>).

**Table 9:** List of identified documents by combination of all databases

Area of interest	N° relevant documents	RIS file	Excel file
Area 1 - Data on chemical identification and characterization of NOCs found in food products, including processing conditions	176	Area_1_Relevant.ris	Area_1_Relevant_formatted
Area 2 - Data on sources and occurrence in food, including human milk	234	Area_2_Relevant.ris	Area_2_Relevant_formatted
Area 3 - Data on toxicokinetics (absorption, distribution, metabolism, excretion) in experimental animals and humans and from <i>in vitro</i> studies	148	Area_3_Relevant.ris	Area_3_Relevant_formatted
Area 4 - Data on toxicity in experimental animals	143	Area_4_Relevant.ris	Area_4_Relevant_formatted
Area 5 - Data on <i>in silico</i> , <i>in vitro</i> and <i>in vivo</i> genotoxicity and mode of action	726	Area_5_Relevant.ris	Area_5_Relevant_formatted
Area 6 - Data on observations in humans (including epidemiological studies, case reports, biomarkers of exposure)	333	Area_6_Relevant.ris	Area_6_Relevant_formatted
All relevant (non-redundant)	1494	All relevant non redundant.ris	

## 4. Conclusions

For this call, an Extensive Literature Search (ELS) as well as the selection of relevant studies by screening of title and abstract related to the chemical identity and characterisation, sources and occurrence in food, toxicokinetics and toxicity of *N*-nitroso compounds (NOCs) in food was performed. In conformity to the need of EFSA, the literature search was organized by following the general principles for systematic reviews as specified by EFSA guidance. The methodology was implemented and tailored in order to address the tasks underlined in the tender specifications in a realistic and well-structured way.

The ELS and the selection of relevant documents allowed us to identify the following relevant articles divided by area of interest:

- Area 1 (Data on chemical identification and characterisation of NOCs found in food products) 176 relevant documents.
- Area 2 (Data on sources and occurrence in food, including human milk) 234 relevant documents.
- Area 3 (Data on toxicokinetics, i.e. absorption, distribution, metabolism, excretion, in experimental animals and humans and from *in vitro* studies) 148 relevant documents.
- Area 4 (Data on toxicity in experimental animals) 143 relevant documents have been identified.
- Area 5 (Data on *in silico*, *in vitro* and *in vivo* genotoxicity and mode of action) 726 relevant documents.
- Area 6 (Data on observations in humans including epidemiological studies, case reports, biomarkers of exposure) 333 relevant documents.

The total number of non-redundant document found was 1494.

## Abbreviations

EC	Exclusion criteria
ELS	Extensive literature search
NOCs	<i>N</i> -nitroso compounds
SSS	Substructure search